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Infusion of Allogeneic 3rd party CD19-specific T cells (CD19RCD137+ T cells) in Patients with refractory CD19+ B-lineage malignancies

Dr. Laurence JN Cooper, MDACC

- To generate CD19-specific T cells that can be infused on demand when the intended recipient needs them, rather than when the T cells are available
- This will be achieved by genetically modifying umbilical cord blood-derived T cells to express a CD19-specific chimeric antigen receptor (CAR) and genetically editing the T cells with engineered artificial nuclease to eliminate expression of T-cell receptor

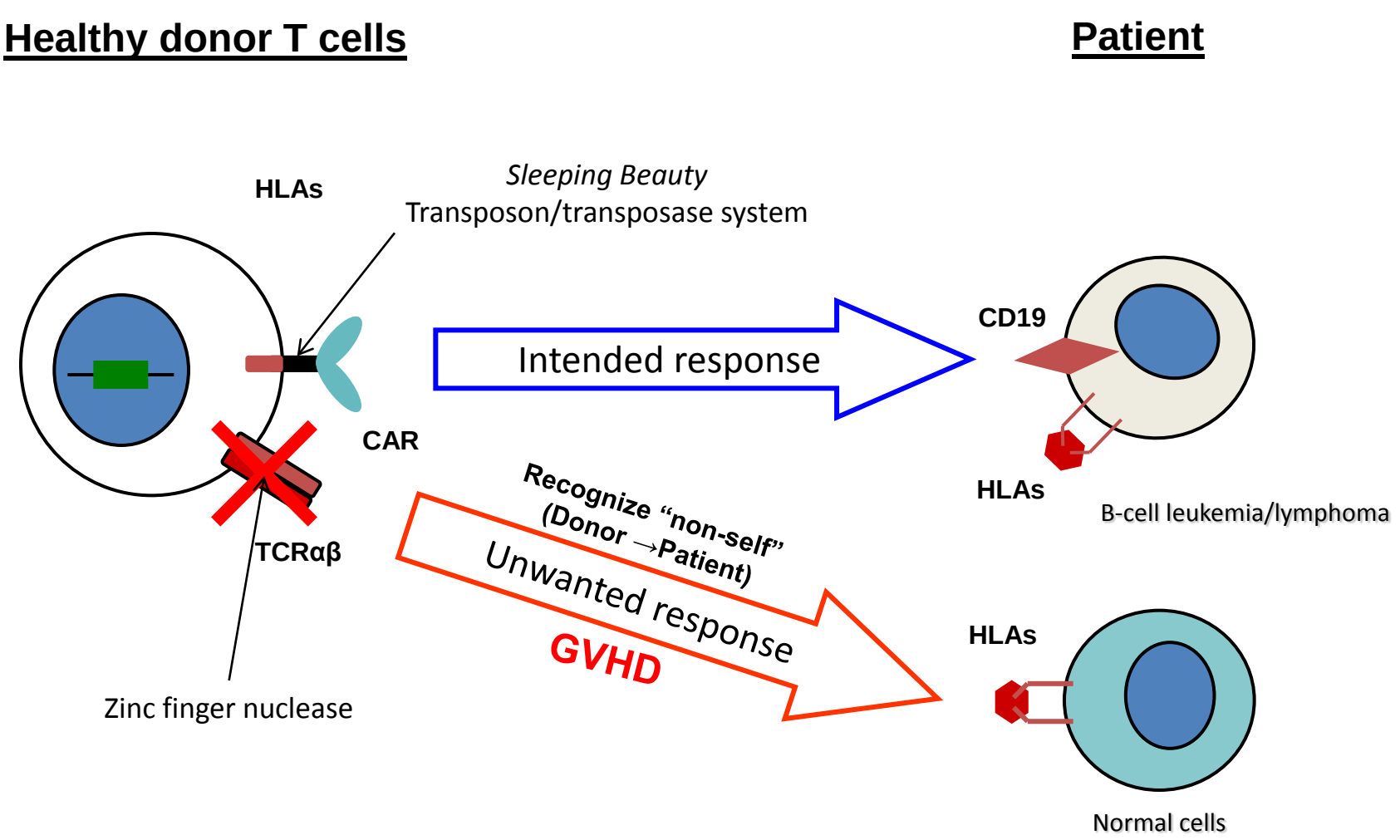
Issues to overcome in investigator-initiated clinical trials in academic centers

- Difficulties in generating CAR^+ T-cells from patient
- Cost and resources to generate CAR^+ T-cells for infusing into a single patient
- Many patients cannot wait for the manufacture and release of autologous CAR^+ T cells
- Many patients' immune system is damaged precluding generation of autologous CAR^+ T cells

Investigator-initiated Gene Transfer Trials under 4 INDs at MD Anderson Cancer Center

MDACC/ NCI #	Agent	Dose of CD19RCD28^{+} T cells	Enrolled	Products made	Infused
2007-0635/ 00968760	CD19-specific T cells derived from patient combined with autologous HSCT	$5x10^{7}/m^{2}$ to $5x10^{9}/m^{2}$ (IL-2 last 2 cohorts)	9 (all NHL)	7	5
2009-0525/ 01497184	CD19-specific T cells derived from donor combined with allogeneic HSCT	$10^{6}/m^{2}$ to $10^{8}/m^{2}$	18 (ALL, n=11; NHL, n=6; CLL, n=1)	14	5
2010-0835/ 01362452	CD19-specific T cells derived from umbilical cord blood (UCB) donor combined with UCB transplantation	$10^{6}/m^{2}$ to $10^{8}/m^{2}$	4 (ALL, n=3; NHL, n=1)	4	1
2011-1169/ 01653717	CD19-specific T cells from CLL patients after chemotherapy (non-HSCT)	$10^{7}/m^{2}$ to $5x10^{10}/m^{2}$	1	1	0

One donor's genetically modified T cells to be infused in multiple patients



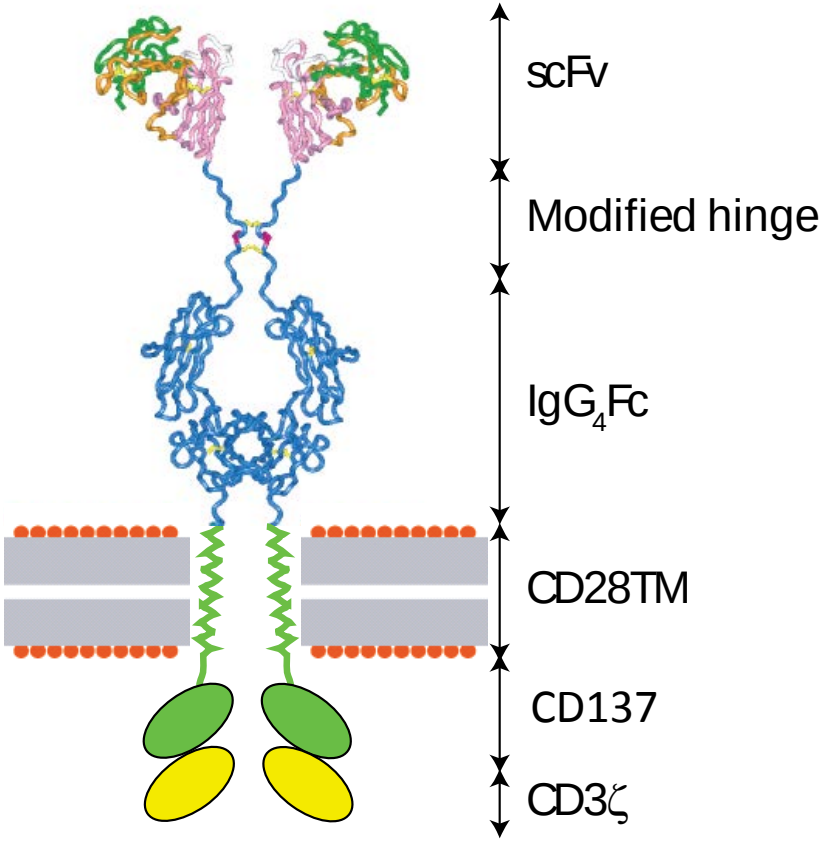
Defining an off-the-shelf T-cell product

- At this point, the desired product will be:
  - CAR^{+}
  - TCR^{neg}
- We recognize, and anticipate, that infused allogeneic (HLA-disparate) T cells may be recognized by endogenous immune system leading to eradication of administered product
  - This may be desirable regarding patient safety for this first-in-human application of $CAR^{+}TCR^{neg}$ T cells
  - Infusion of HLA-mismatched T cells occurs in other clinical settings (after HSCT) and results in therapeutic responses
  - We have plans to use ZFNs to generate $CAR^{+}TCR^{neg}$ HLA^{neg} T cells

SB system to genetically modify T cells to target CD19

- T cells targeting CD19 feasible gene therapy approach
  - Successful infusions of genetically modified T cells
  - Tolerable “on target” side-effects
- Compelling patient population
  - Patients with advanced B-cell malignancies high rate of relapse

2nd generation CD19-specific CAR (**CD19RCD28**) signaling through CD28 and CD3-ζ



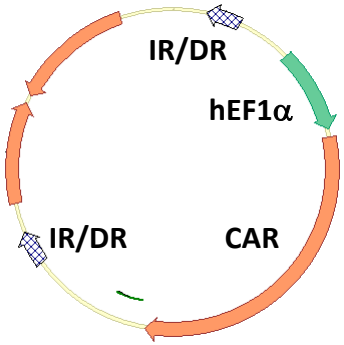
Shown as a homo-dimer

Approach to manufacturing

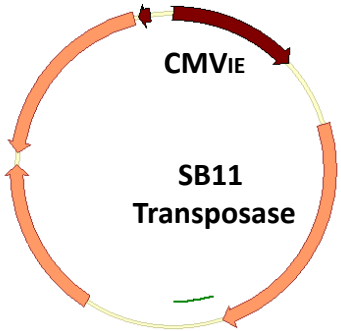
- UCB from banks collected in compliance with current good manufacturing practice
- T cells electroporated with SB system to stably express 2nd generation CAR targeting CD19
  - CD19RCD137 CAR activates via chimeric CD3-ζ and CD137
- T cells electroporated with *in vitro*-transcribed mRNA coding for ZFNs specific for TCR β
- T cells propagated on aAPC and cryopreserved as a bank
  - Maintains telomere length to preserve replication senescence

Sleeping Beauty (SB) system transposon/transposase

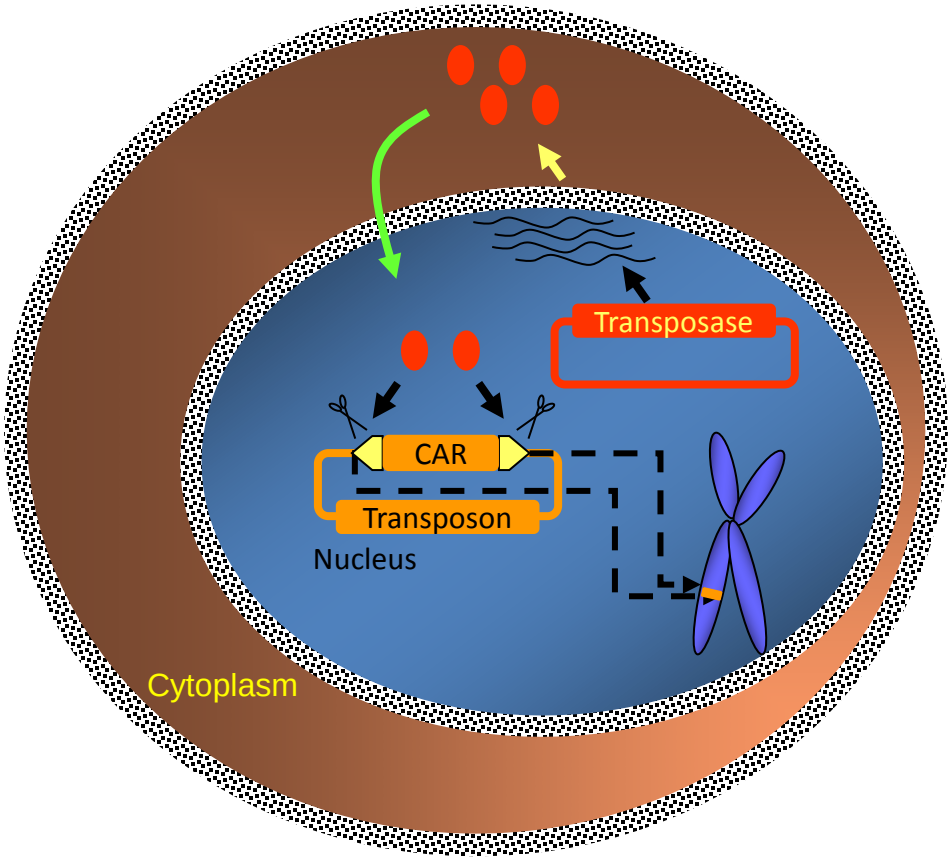
Transposon DNA plasmid



Transposase DNA plasmid
(or *in vitro* transcribed mRNA)

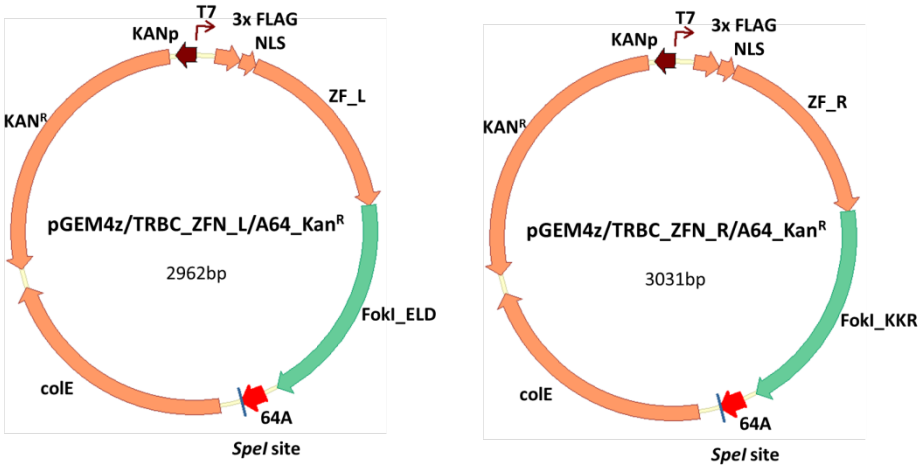


Co-delivery into cells by nucleofection (Lonza)



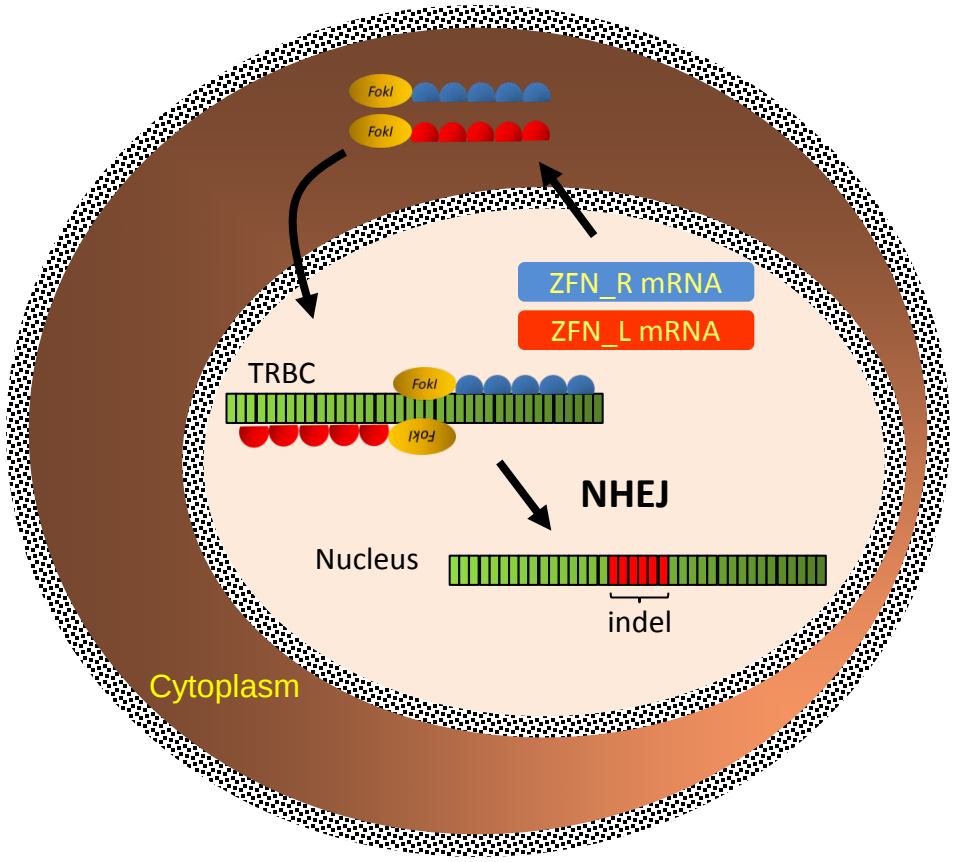
ZFNs targeting TCR β

ZFN DNA plasmids
for *in vitro* transcription to generate ZFNs

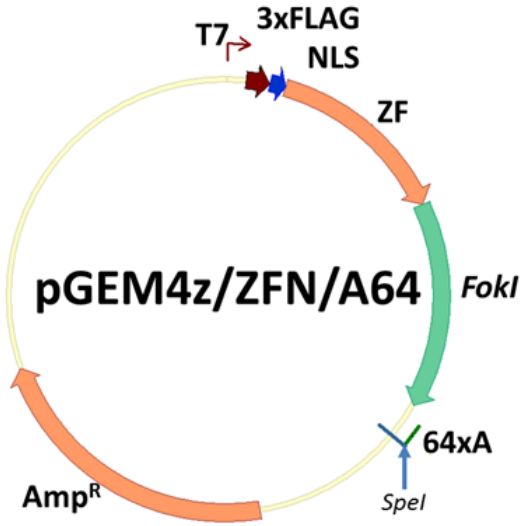


In vitro transcription

Co-delivery into cells by nucleofection (Lonza)



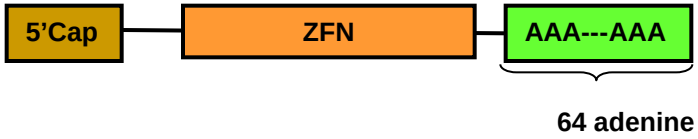
In Vitro Transcription of mRNA From Template Plasmid



Linealized by *SpeI* → **Overnight digestion**

T7 RNA polymerase
Cap Analogue → **2-3 hours Reaction**

Amplified, in vitro transcribed mRNA

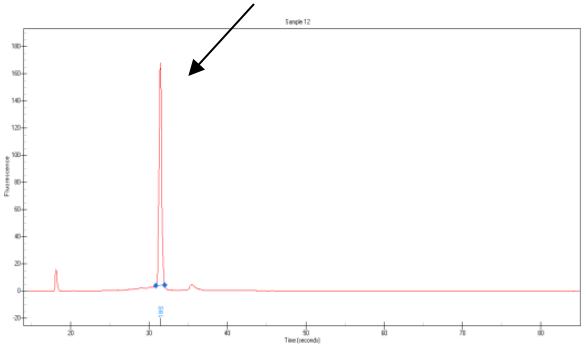


64 adenine

Release criteria

- ✓ Single peak of expected size on BioAnalyzer
- ✓ A260/230 > 1.8
- ✓ A260/280: 1.8-2.1
- ✓ Negative bacteria and fungal cultures

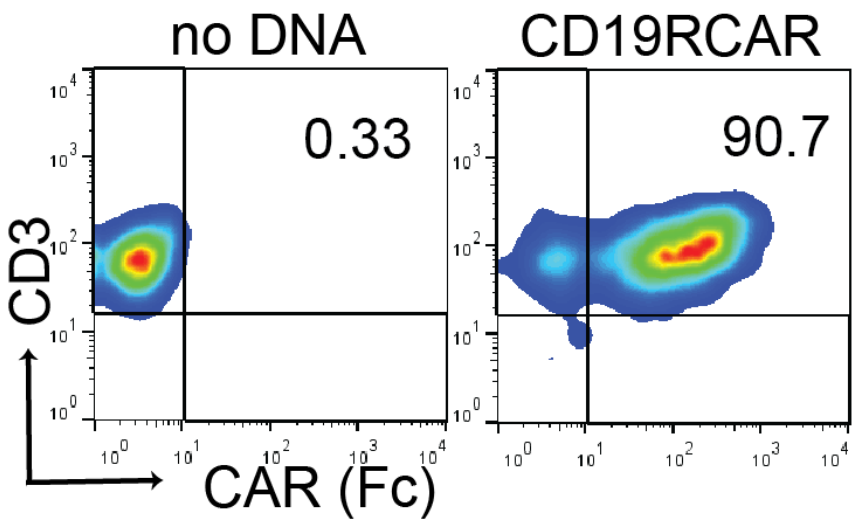
Single peak



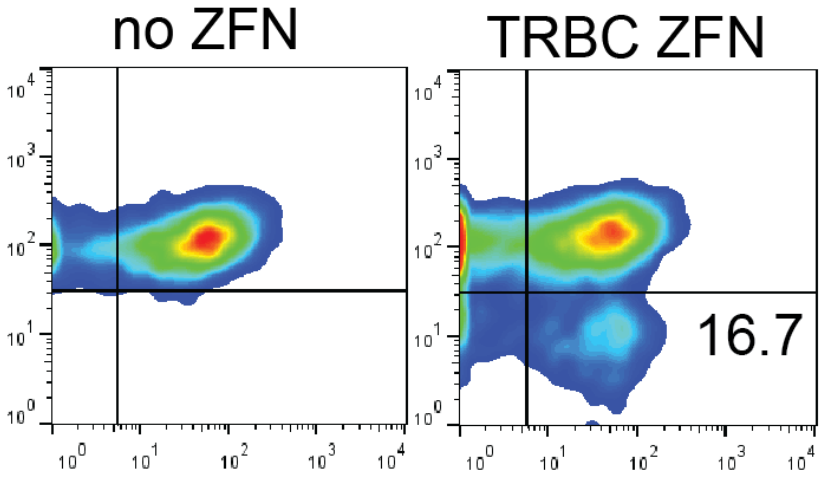
Bioanalyzer

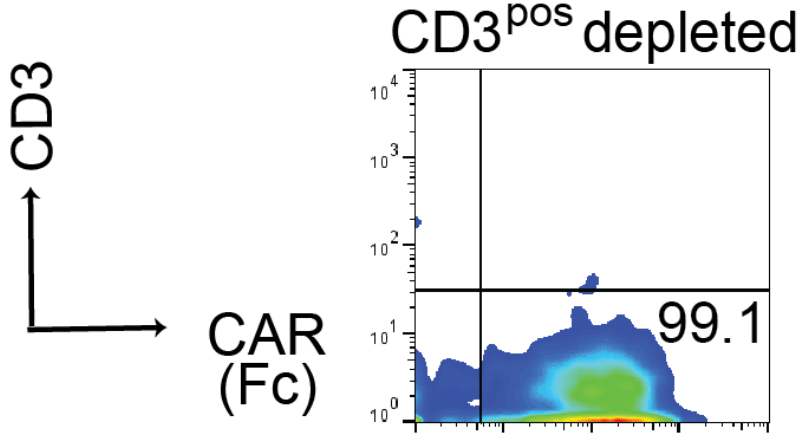
Off-the-shelf CD19-specific CAR^{+} T cells from UCB

Day15 after CAR DNA +SB11 DNA



Day6 after electro-transfer TRBC.ZFN from mRNA





Path to the clinic

- SB system has been used to genetically modify T cells to express CD19-specific CAR
- Patients have received SB-modified CAR^+ T cells derived from UCB and peripheral blood
- Approach to manufacturing can be adapted to electroporate DNA and mRNA to stably express CAR and eliminate TCR

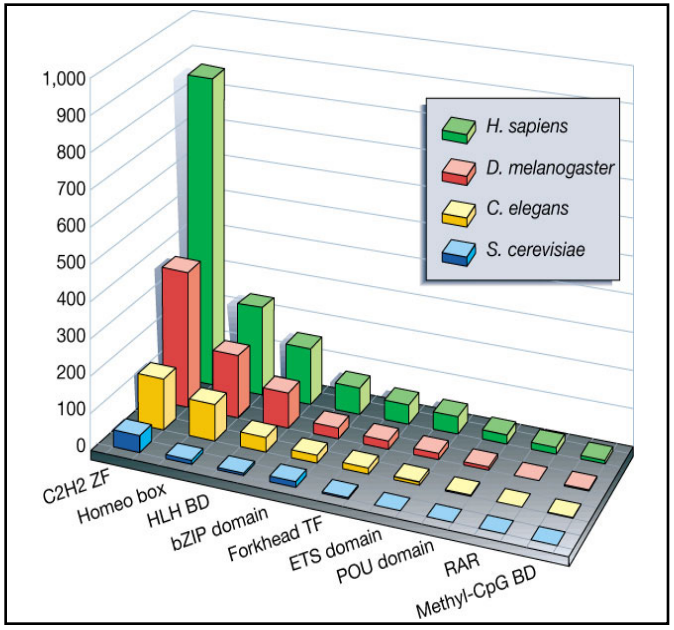
Zinc Finger Nucleases Targeting TCRβ

Infusion of Allogeneic 3rd party CD19-specific
T cells (CD19RCD137+ T cells) in Patients with refractory CD19+ B-lineage malignancies

Dr. Philip Gregory, Sangamo BioSciences

What are Zinc Finger Proteins (ZFPs)?

DNA binding motifs,
abundance by species



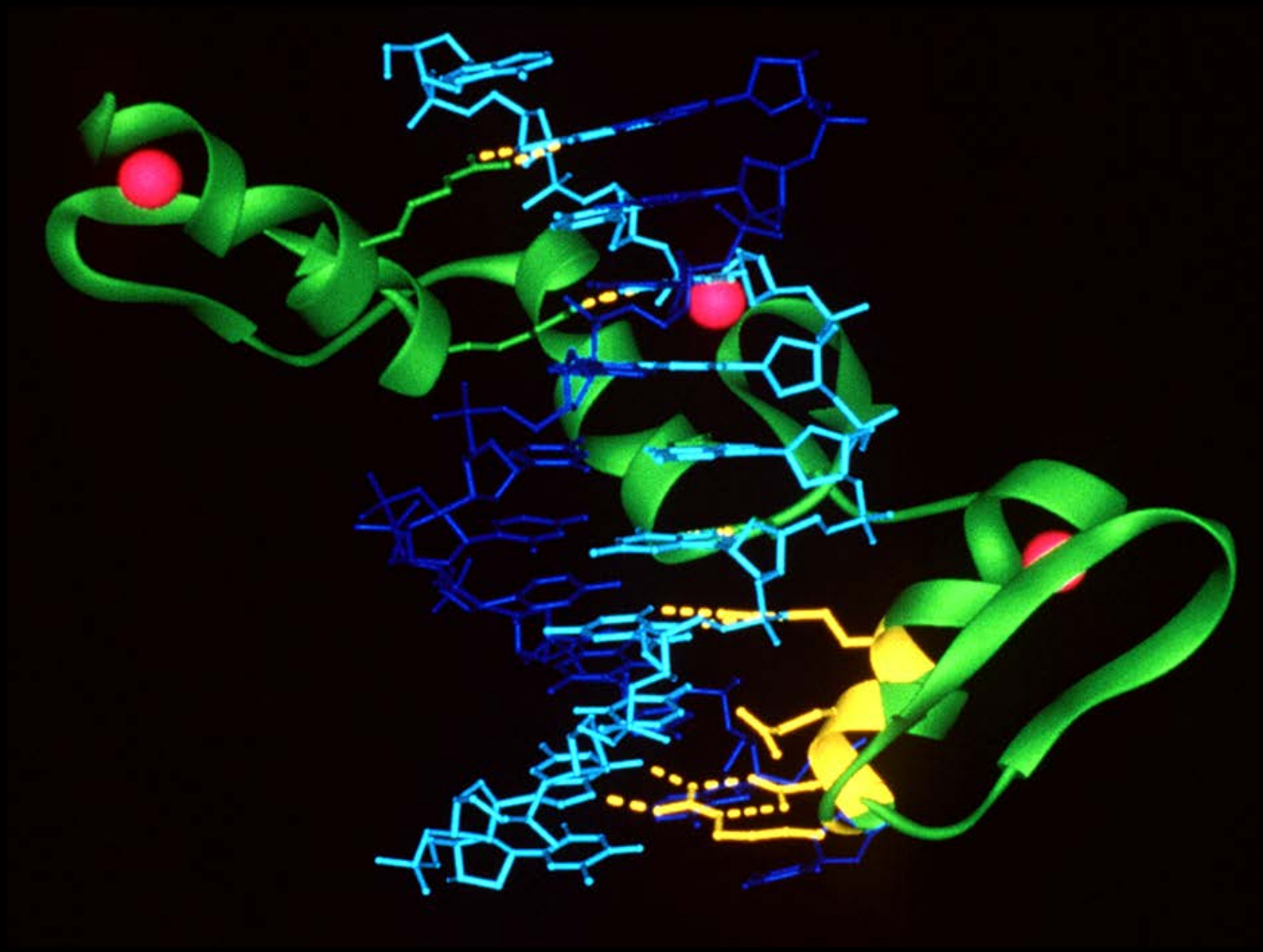
Tupler R, Perini G, Green MR (2001). *Nature* 409: 832-833.

- Natural class of DNA binding proteins / transcription factors
- Exhibit sequence specific binding to a broad array of DNA sequences
- By far the most abundant class of DNA-binding domains found in human transcription factors

Natural versatility and specificity of ZFPs enables application to therapeutic target genes

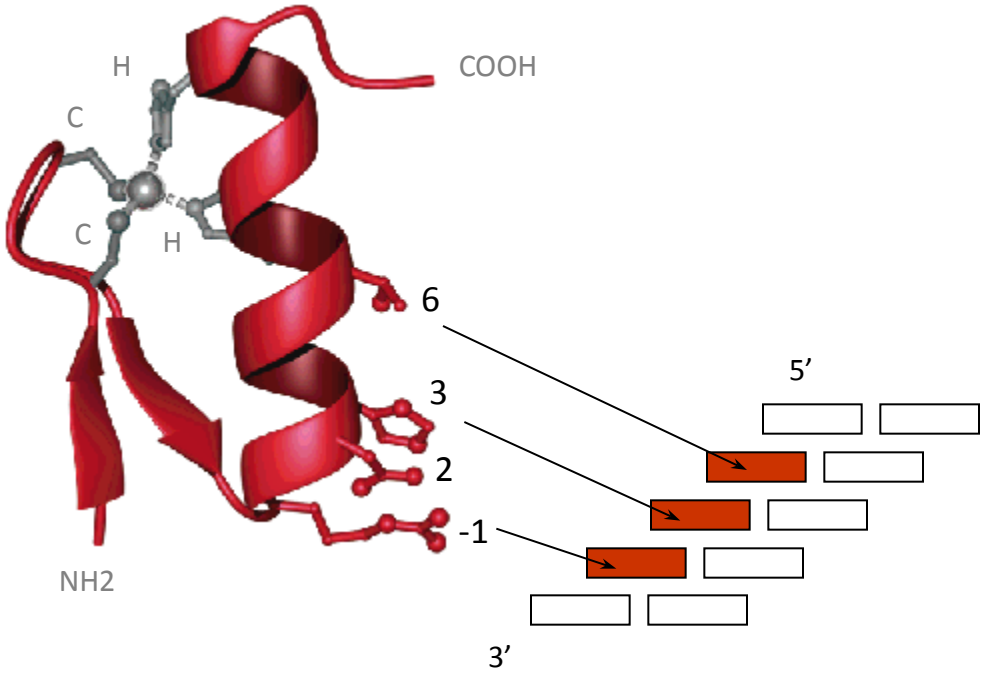
Principles of ZFP-DNA Recognition

- Tandem fingers in major groove
- Each finger binds 3-4 bp
- N-finger protein spans 3N bp
- Target sequence is composite of finger subsites



Zif 268 - DNA complex; coordinates from
N.P. Pavletich and C. O. Pabo, *Science* 252, 809 (1991)

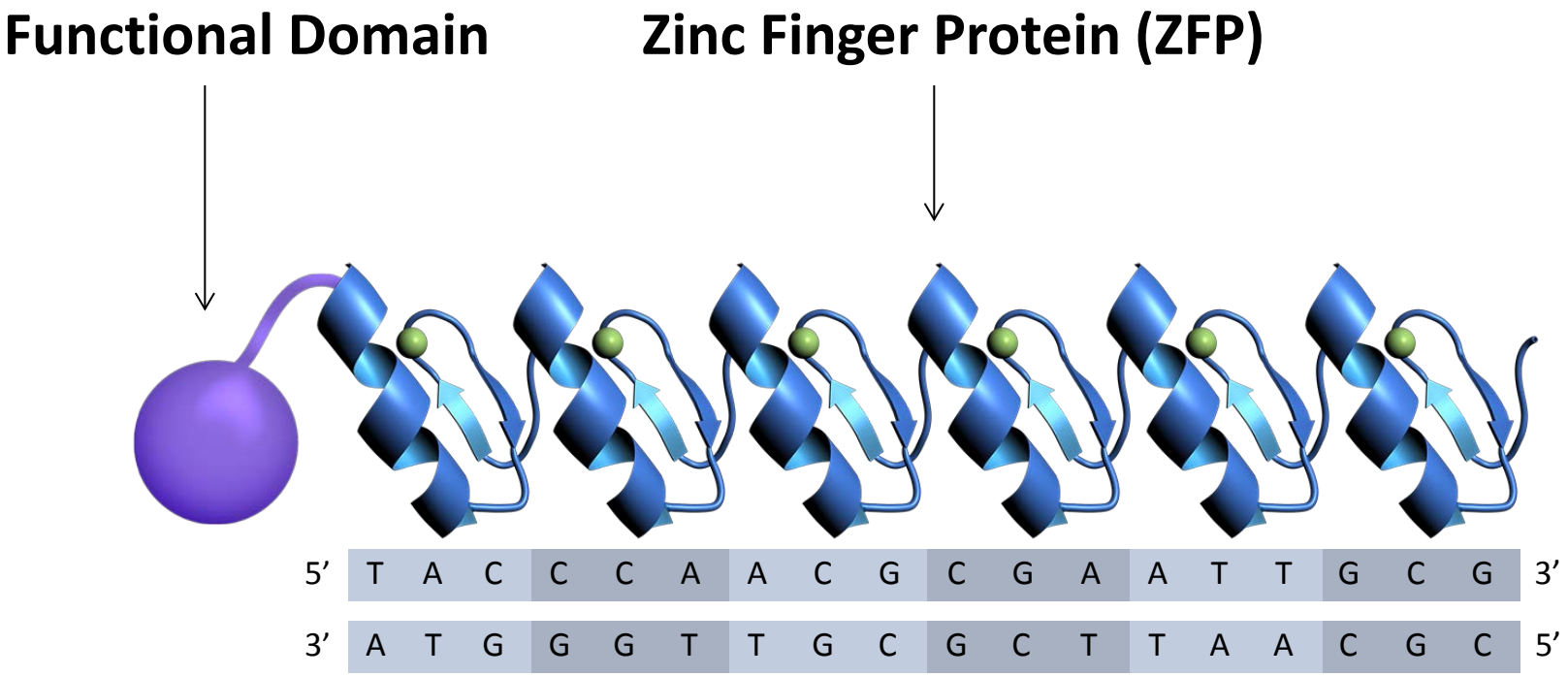
DNA Recognition By Zinc Fingers



Finger "Design" -1 2 3 6	Triplet Specificity
S LT	GCG
S LT	GGG
S LT	GCT
S LT...	GCA...

- Mediated by residues -1 to 6 of α-helix
- Most critical positions are -1, 2, 3 and 6
- One finger typically contacts 3 bases
- Correspondence between helix sequence and triplet binding preference

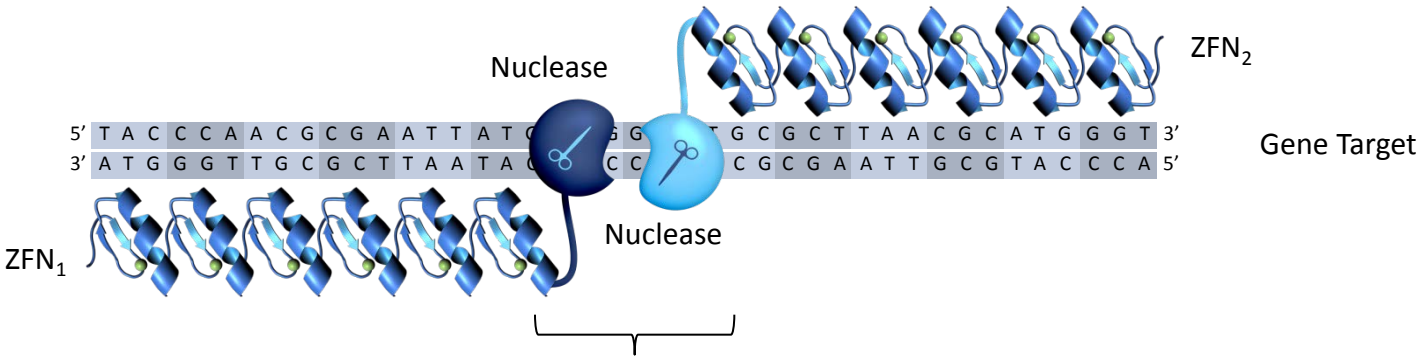
ZFPs are linked to functional domains to drive targeted therapeutic outcomes



ZFP Nucleases (ZFNs) edit genes

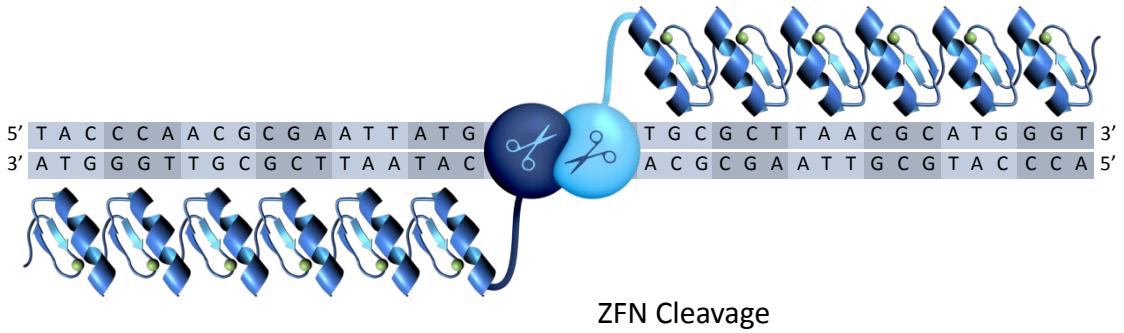
Gene Target

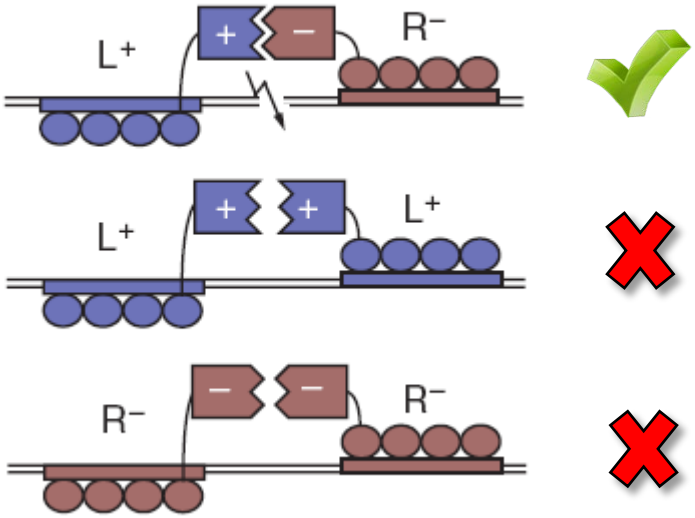
ZFP Nucleases (ZFNs) edit genes



Modified nucleases (obligate heterodimers) further increases specificity

ZFP Nucleases (ZFNs) edit genes





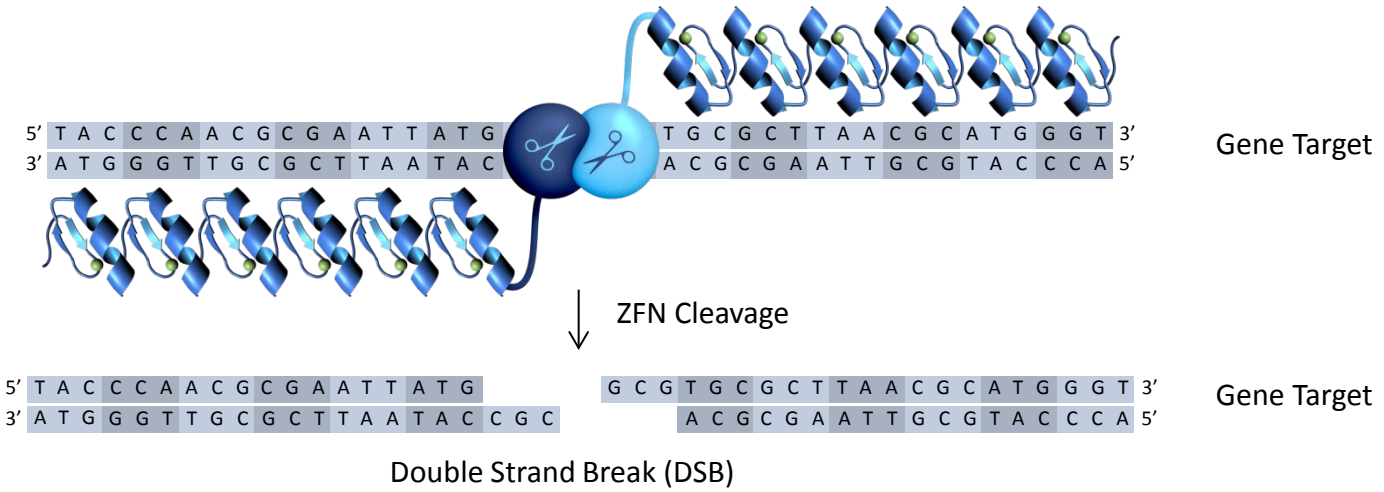
Miller et al. NBT 2007

An improved zinc-finger nuclease architecture for highly specific genome editing

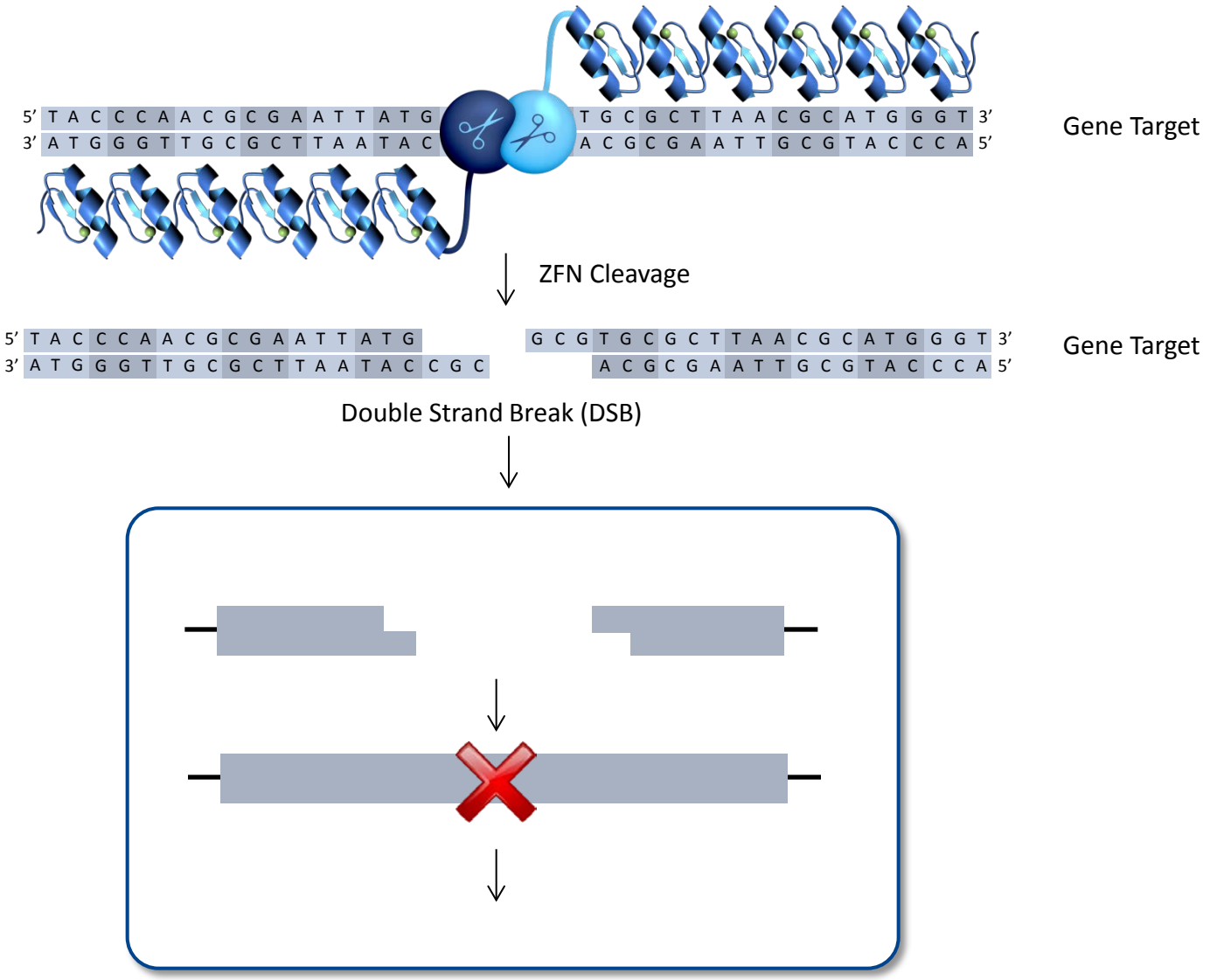
Doyon et al. NMETH 2010

Enhanced zinc-finger nuclease activity with improved obligate heterodimeric architectures

ZFP Nucleases (ZFNs) edit genes

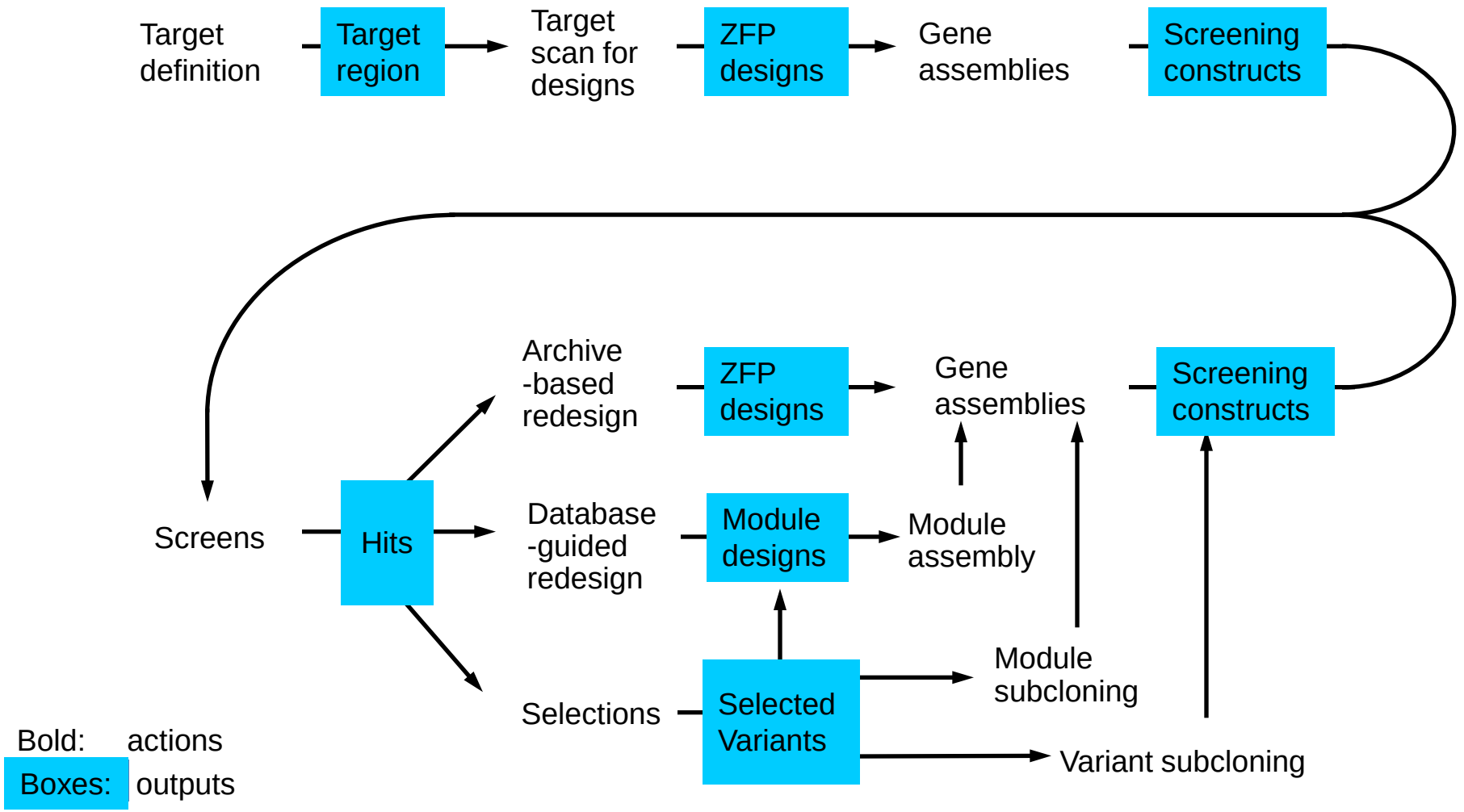


ZFP Nucleases (ZFNs) edit genes



NHEJ = Non-homologous End Joining

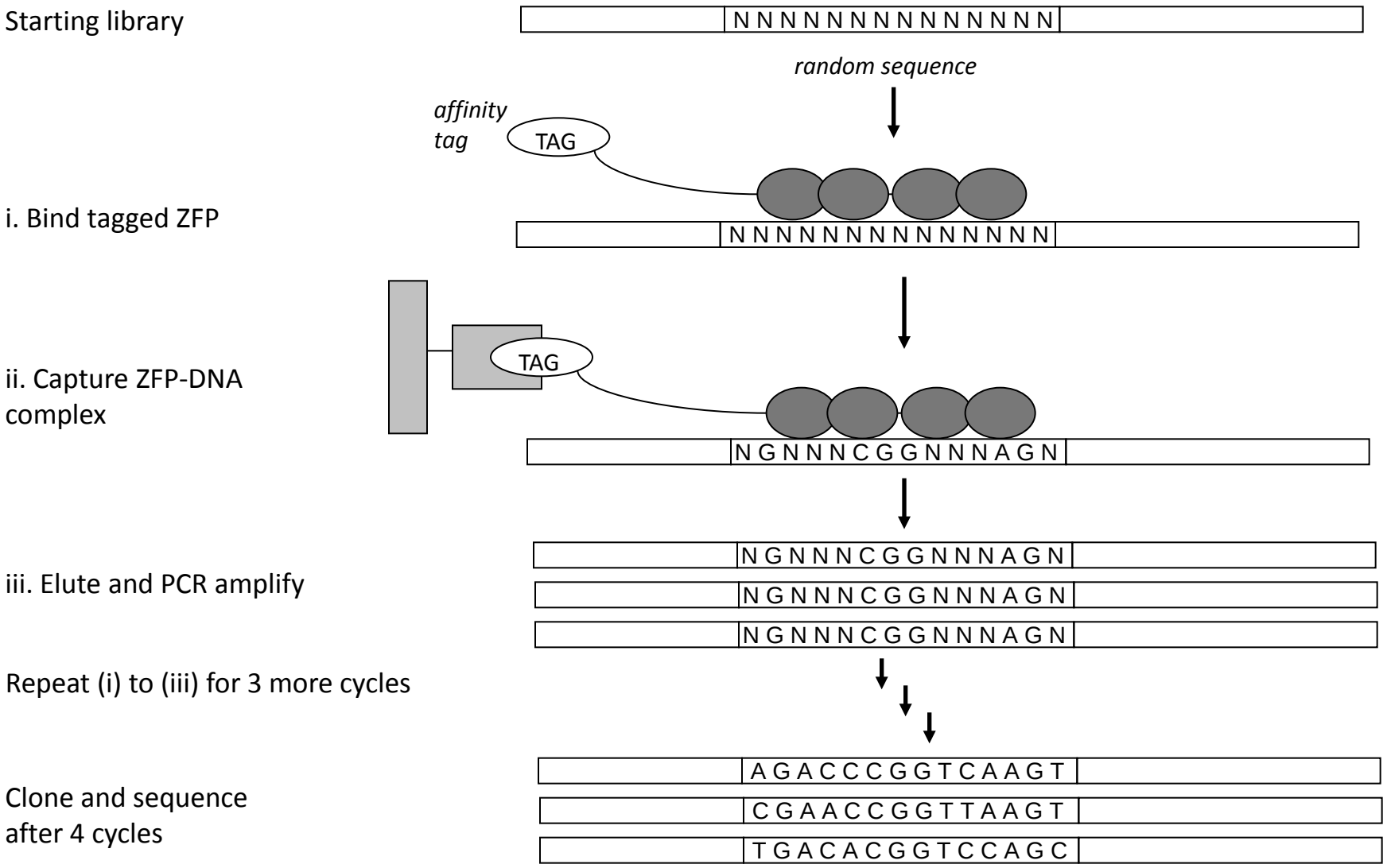
Lead development overview



Factors affecting ZFN specificity

- High DNA binding specificity intrinsic to wt and engineered C_2H_2 zinc finger DNA binding domains
- The absolute requirement for both ZFN monomers to be resident on the DNA target to generate an active nuclease with catalytic activity
- The dimerization interface of the FokI domain is weak resulting in independent monomer DNA binding
- Use of obligate heterodimer FokI domains which reduce the possibility of unwanted cleavage by homodimerization
- Strict spatial / orientation requirements for cleavage
- Fidelity of the natural DNA repair pathways

Site Selection Assay (SELEX)



Assessing the specificity of ZFN action

Molecular assessment for ZFN specificity:

- SELEX-based analysis of the top 30 sites genome-wide with the highest homology to the experimentally determined consensus
- Samples chosen for analysis will use an ZFN mRNA dose in excess of that used in the clinical manufacturing process
- Direct DNA sequencing assay (run at ≥10,000 sequence reads per site)

*Note that the selection for cells lacking CD3 (a result of **on target** activity) further serves to increase the sensitivity of this off-target analysis*

Biological assessments:

- Demonstrate the lack of autonomous growth in the absence of activation signaling and exogenous cytokines (IL-2)
- Confirm normal karyotype of the final product

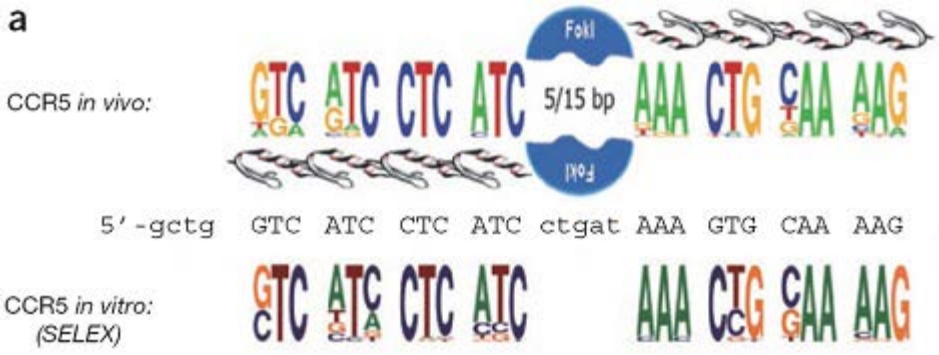
Other assays for ZFN specificity

Pattanayak, V. *et al.*. Revealing off-target cleavage specificities of zinc-finger nucleases by *in vitro* selection. *Nat. Meth.* 8, 765–770 (2011).

- *In vitro* system with limited utility due to excess of ZFN used
- Sites identified have minimal overlap with known cleavage events *in vivo*

Gabriel, R. *et al.* An unbiased genome-wide analysis of zinc-finger nuclease specificity. *Nat. Biotechnol.* 29, 816–823 (2011).:

- *In vivo* approach – laborious but useful (more suited to high risk applications)
- Sites identified have been confirmed by independent methods
- Data support the consensus binding activity derived by SELEX
- <u>SELEX-based off target search is a facile and sensitive method for ZFN evaluation</u>



in vivo LAM-PCR

in vitro SELEX

Preliminary application of ZFNs targeting TCRβ

No off-target cleavage at the SELEX derived Top 15 sites*

nature medicine

Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer

Elena Provasi[1,2,11], Pietro Genovese[2,3,11], Angelo Lombardo[3], Zulma Magnani[1], Pei-Qi Liu[4], Andreas Reik[4], Victoria Chu[4], David E Paschon[4], Lei Zhang[4], Jurgen Kuball[5,10], Barbara Camisa[1], Attilio Bondanza[1], Giulia Casorati[6], Maurilio Ponzoni[7], Fabio Ciceri[1,8], Claudio Bordignon[2,9], Philip D Greenberg[5], Michael C Holmes[4], Philip D Gregory[4], Luigi Naldini[2,3] & Chiara Bonini[1,2,8]

No off-target cleavage at the SELEX derived Top 15 sites*

blood

A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR

Hiroki Torikai,[1] Andreas Reik,[2] Pei-Qi Liu,[2] Yuanyue Zhou,[2] Ling Zhang,[1] Sourindra Maiti,[1] Helen Huls,[1] Jeffrey C. Miller,[2] Partow Kebriaei,[3] Brian Rabinovitch,[1] Dean A. Lee,[1,4] Richard E. Champlin,[3] Chiara Bonini,[5] Luigi Naldini,[6] Edward J. Rebar,[2] Philip D. Gregory,[2] Michael C. Holmes,[2] and Laurence J. N. Cooper[1,4]

* Assay sensitivity ~1% (on target activity ~48%)

NIH RAC experience with ZFN-modified cells

Specificity assessment exploited SELEX-based Top 15 sites

0704-843 *A Phase I Study of Autologous T-Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nuclease SB-728 in HIV-Infected Patients.*

Important features: HIV, adenoviral (Ad5/F35) vector, autologous CD4+ T cells, ZFN KO

NIH/OBA Receipt Date: 4-13-07. **Publicly Reviewed at the June 2007 RAC meeting**

0704-848 *A Phase I Study of Intratumoral Administration of Cellular Immunotherapy for Recurrent/Refractory Malignant Glioma Using Alloclone-002 Modified for Glucocorticoid Resistance and Interleukin-2.*

Important features: GBM, adenoviral vector (Ad5/F35), allogeneic CD8+ T cells, ZFN KO

NIH/OBA Receipt Date: 4-20-07. **Publicly Reviewed at the June 2007 RAC meeting**

1304-1228 *A Phase I Study of Autologous T- Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases SB-728 in HIV Infected Patients Pre-treated or Not with Cyclophosphamide.*

Important features: HIV, mRNA electroporation, autologous CD4+ T cells, ZFN KO

NIH/OBA Receipt Date: 04-15-13. **Not Selected for RAC Public Review: 05-07-13**

Patient safety

Molecular assessment for ZFN specificity:

- SELEX-based analysis of the top 30 sites genome-wide with the highest homology to the experimentally determined consensus
- Samples chosen for analysis will use an ZFN mRNA dose in excess of that used in the clinical manufacturing process
- Direct DNA sequencing assay (run at ≥10,000 sequence reads per site)

Biological assessments:

- Sterility
  - Bacteria, fungi, mycoplasma, endotoxin
- Identity
  - CAR expression, CD4/CD8 expression
  - Lack of TCR/CD3, CD32 (aAPC)
  - HLA expression
- Safety
  - Demonstrate the lack of autonomous growth in the absence of activation signaling and exogenous cytokines
  - Confirm normal karyotype
  - Lack of SB11

Genetic engineering is needed to meet patient needs

- T cells genetically modified to express CAR and eliminate TCR can be pre-made and infused on demand into multiple recipients
- The final product will be manufactured at MDACC
- Trial undertaken to evaluate safety and feasibility of this approach to T-cell therapy in a patient population with unmet clinical needs

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Making Cancer History®

Phase I Clinical Trial overview

Infusion of Allogeneic 3rd party CD19-specific T cells (CD19RCD137+ T cells) in Patients with refractory CD19+ B-lineage malignancies

Dr. Partow Kebriaei, MDACC

Rationale

- Patients with advanced CD19 positive lymphoid malignancies are refractory to all currently available treatment, including high dose chemotherapy and hematopoietic stem cell transplant.

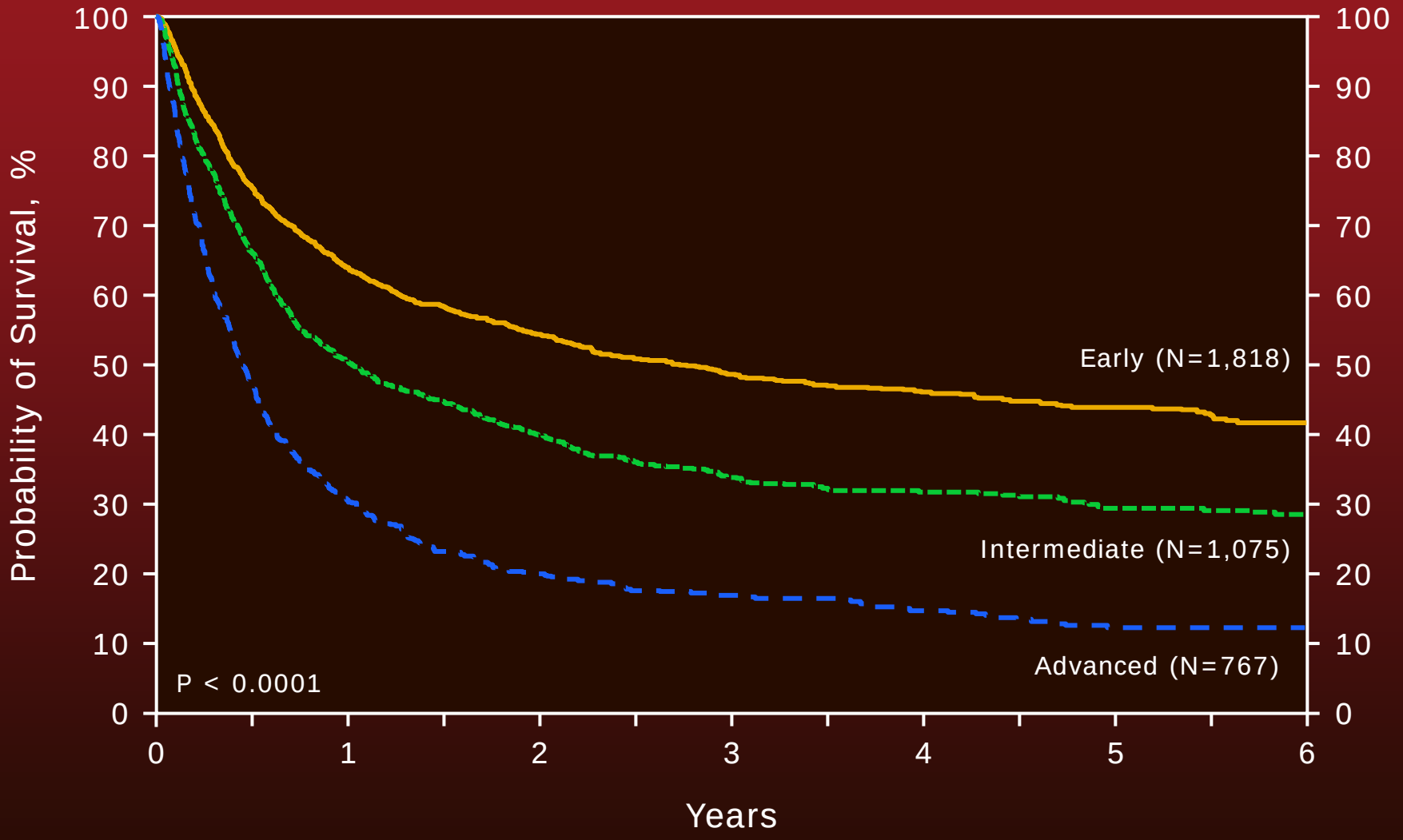
* Treatment needed urgently at time of relapse.

Probability of Survival after HLA-identical Sibling Transplants for Diffuse Large B-Cell Lymphoma, 2000-2010

- By Disease Status -

Probability of Survival after Unrelated Donor Transplants for ALL, Age ≥ 20 yrs, 2000-2010

- By Disease Status -



Protocol Objectives

- Safety, feasibility, and persistence of 3rd party, modified T cells
  - Host immune response against CAR
  - Homing ability of genetically modified T cells
  - Disease response

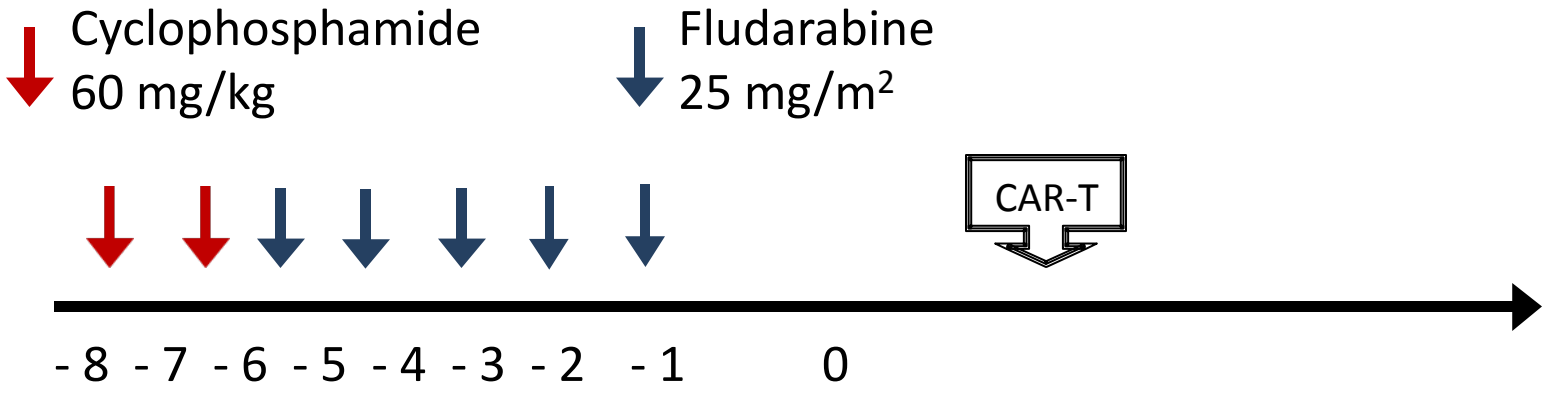
Rationale

- Enhance immune response to improve survival
  - Target disease using mechanisms independent of chemo- and radiotherapy with non-overlapping toxicities
  - Genetically modified, tumor-specific T cells
  - Readily available product

Study Population

- Refractory or relapsed $CD19^{+}$ lymphoid malignancies, CLL, ALL, NHL, SLL, FL or MCL, not in complete remission
- Patients must be minimum 3 months from prior transplant
- Age 18-70 years
- Zubrod performance 0–2, or Lansky PS $\geq$ 50%
- No active infection or Grade >2 (CTC vs. 4) toxicity at time of T-cell infusion

Treatment Plan



- T cells infusion no sooner than 48 hours and no later than 1 week post completion chemotherapy.
- T-cell infusion over 2 days: Up to 25% of cells first day, and up to 75% of the remaining T cell on second day.
- Patients eligible for recurrent therapy with lymphodepletion followed by T cell infusion up to three times if remain with active disease, with minimum 6 weeks of observation between treatments.

Statistical Design

Dose Cohort	Single T-cell Dose
Dose Level -1	Not to exceed $10^6/m^2$
Dose Level A	$>10^6/m^2$ but $\leq 10^7/m^2$
Dose Level B	$>10^7/m^2$ but $\leq 5 \times 10^7/m^2$
Dose Level C	$> 5 \times 10^7/m^2$ but $\leq 10^8/m^2$
Dose Level D	$>10^8/m^2$ but $\leq 5 \times 10^8/m^2$
Dose Level E	$> 5 \times 10^8/m^2$ but $\leq 10^9/m^2$
Dose Level F	$>10^9/m^2$ but $\leq 5 \times 10^9/m^2$

- Max 42 patients enrolled at 6 dose combinations
- Study duration 1 year
- Descriptive: demographics, clinical events/dose level
- Number of patients with DLT/dose level
  - Dose escalate if < 2/3 of patients have DLT

Evaluations, Safety Assessments

- Pre-Treatment
  - Baseline disease staging
  - Well-described release criteria for T-cell product
  - B- and T-cell subset analyses by flow, quantitative Igs, baseline serologic testing for antibody response against CD19-specific transgene
- After T-cell infusion
  - Cultures of T-cell product obtained to monitor for contamination
  - Toxicity grading for adverse events
  - Testing for protein expression and genetic typing, including PCR for presence of infused T cells and assessment of clonality
  - Disease staging
- Long-term follow-up
  - Yearly safety evaluations for 15 years for patients who received T-cell infusion

Management of Potential Toxicity

- Microbial contamination of T-cell product
  - Standard GMP procedures will be followed for microbial contamination
- Management of adverse event (AE) attributable to T cells
  - Start tocilizumab
  - Then, start corticosteroids
  - If no improvement additional immunosuppression added

Preparing for multi-center trials

- Patient-specific T cells teach proof-of-principle, but inherent heterogeneity complicates translational appeal
- We didn't have to make tumor-specific T cells for every patient, but instead could make one source of T cells for all patients?
  - Lends itself to centralized manufacturing
  - Undertake Phase IIb multi-center trials powered for efficacy
  - Undertake multi-component trials at T-cell MTD